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THE RELATION OF CRITHIDIA MELOPHAGIA TO THE SHEEP'S BLOOD, WITH REMARKS UPON THE CONTROVERSY BETWEEN DR. PORTER AND DR. WOODCOCK.*

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Recently Miss Porter (1911) published in *Parasitology* a criticism on Woodcock's work in the *Zoological Record* for 1909. The criticism was against his action in inserting what she held to be his own opinions on the genera *Crithidia* and *Herpetomonas,* which are (quoting from her) "extreme and decidedly opposed to the opinions of those who have worked personally on the parasites in question."

In Woodcock's reply (1911) to her complaint against his putting the generic names, *Crithidia* and *Herpetomonas* in inverted comas, he states that he placed in inverted comas only the *Crithidia* and *Herpetomonas* from *blood-sucking* hosts; but in the case of *Crithidia* and *Herpetomonas* from *non-blood-sucking* hosts, he did not use inverted comas—that is, he left these as valid genera.

In Miss Porter's paper I was mentioned as one who did not agree with this view of Woodcock. In replying Woodcock used certain open-minded statements of mine, taken from a letter to him and from a footnote on page 141 of my paper (1911), in support of his view (hypothesis) that *Crithidia* and *Herpetomonas* from all *blood-sucking* insects "are in all probability not to be regarded as belonging to truly independent genera but are merely developmental phases of some Trypanosome." (Woodcock 1911, page 154.) The fact is that both in my footnote and letter reference was made specifically, and *only,* to *Crithidia melophagia,* and I was not even open-minded to such a general hypothesis. Many other statements in the paper would show this to be true; but most of all perhaps the fact that in the same paper I described what I called a *true Herpeto-*

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monas from the rat-flea, stating that rat-fleas harbor pure *Herpetomonas,* pure trypanosomes or a mixture of the two. In Miss Porter's reply (1911a) to Woodcock, she has adequately defended our side of the question.

The reason I was open-minded to the idea that *C. melophagia* might possibly be a phase of the life history of a sheep trypanosome, was the facts that Woodcock (1911), after a prolonged search, discovered a trypanosome in the sheep's blood, and that it is now known that certain trypanosomes change over into crithidia-like forms in their insectan hosts. To be sure, no one had been able previously to find a flagellate in the sheep's blood, altho many sheep, which were heavily infected with sheepticks containing *C. melophagia,* had been examined. Yet, in the case of negative results there still remains a possibility that a further search will yield positive results. And so in the case of the sheeptick flagellate it was not unscientific to admit the possibility that the trypanosome discovered by Woodcock might yet be proved to be connected with *C. melophagia.* And I doubt not that some of the forms from blood-sucking insects, described as true *Crithidia* may later be shown to be only phases of trypanosome cycles. I suggested this in the case of *C. ctenopthalmi.* Anyone comparing Patton and Strickland's description and figures of this parasite with my paper (1911) on the rat trypanosome in the flea will be impressed by the similarity of the parasites. (See Patton and Strickland, 1908, pp. 333-335.) For example, their figure 4 is almost exactly the same as my figures of the small trypanosome appearing in the life-cycle of *T. lewisi.* And so, I believe it may later be shown that *C. ctenopthalmi* is not a valid species, but merely a part of the life cycle of *T. lewisi.* Likewise, it may later be shown experimentally that the form from the rat-flea, described by me (1911) as *Herpetomonas pattoni,* is a phase in the life-cycle of *T. lewisi.* Already, Swellengrebel and Strickland (1911) in their criticism of my paper contend, altho they do not prove, that these forms belong to the trypanosome cycle. Be that as it may, nevertheless I cannot accept Woodcock's hypothesis that the *Crithidia* and *Herpetomonas* from *all blood-sucking* hosts are merely developmental phases of some trypanosome.

Upon receiving Woodcock's reply to Miss Porter, I at once set about to test his hypothesis experimentally in one case, *C. melophagia.*

The method of attack is as follows:

1st. Examine microscopically the blood of several lambs and sheep harboring sheep ticks infected with *C. melophagia.*

2nd. (a) Inject lambs hypodermically, intravenously and intraperitoneally with the contents of the digestive tracts of sheep ticks containing flagellates; then examine the blood for trypanosomes. (b) Feed lambs on sheep ticks and their excrement teased in salt solution.

3rd. Place freshly hatched ticks on a sheep which has been washed in an antiseptic solution, to eliminate the possibility of their getting the flagellates by the ingestion of contaminated faeces. The value of this experiment may be stated thus: If the ticks do not develop flagellates we know that they are not present in the blood of the sheep, for practically 100% of the ticks of the United States under normal conditions develop flagellates. But if they do develop them they must either get them hereditarily or from the blood.

In respect to the first method, others, Flu (1908) and Porter (1910), as well as myself (1908 and 1909), have failed to find any flagellate or in fact any other form in the sheep's blood that could possibly have any connection with *C. melophagia,* altho repeated examinations were made of sheep harboring infected ticks. In my own papers detailed accounts are given of my extensive, but fruitless, attempts to discover a trypanosome or a non-flagellate stage of some parasite from which *C. melophagia* might be derived. Notwithstanding these former results, I have laboriously examined microscopically the blood of thirty sheep and lambs, many of which were harboring infected ticks. Undoubtedly all had harbored them before they were dipped a few weeks earlier, because the flock was heavily infected with ticks. Moreover, the ticks infecting the sheep were without doubt parasitized with *C. melophagia,* for I have not been able to find an uninfected tick in the flock. Generally five slides of of blood (using large cover glasses) from each sheep were carefully examined. Results were always negative. These facts in themselves constitute good evidence against *C. melophagia* being derived from a trypanosome in the blood of the sheep.

The second method yielded additional evidence no less valuable. Lambs were selected for the experiments, for the reason that old sheep might have been infected when young, and have developed an immunity against the flagellates. Such is apparently the case with rat trypanosomiasis. Four lambs about five months old were selected. They were first examined for flagellates, but with negative results, altho they were heavily infected with ticks harboring *C. melophagia.* The youngest, No. 1, probably not more than four months old, was inoculated subcutaneously August 22nd with the contents of the digestive tracts of six sheepticks. In preparing the contents for inoculation the viscera were removed and teased in normal salt solution. The heads and probosces of the ticks were also teased in the same mixture. Examination of the mixture showed it to be teeming with flagellates. These were then injected subcutaneously.

Examinations of the blood were made August 23, 24, 25, 26, 28, and 29. The results were negative.

On August 29th a similar preparation of ten ticks was made. Lambs 507, 606, and 572, each received 1/3 of the mixture, the first two subcutaneously, the last intraperitoneally. At the same time 25 ticks together with masses of excrement were ground up in salt solution and fed to all four lambs by drenching. Examinations of their blood were made as follows: Lambs 1 and 507, Aug. 31; lamb 1, Sept. 1; lambs 572, 507 and 606, Sept. 2; all of them, Sept. 4, 6, 8, 11, 15, 22. September 15th lamb 507 showed a great many bacteria in the blood. Sept. 17th none could be found. Otherwise the results were all negative.

On Sept. 27th the contents from the digestive tracts of 25 ticks were injected directly into the jugular vein of lamb No. 1. Judging from a microscopical examination of the mixture, not less than 1,000,000 flagellates were injected. The blood of the lamb was examined in 10 minutes and again in 20 minutes. On the following day it was again examined. The results were negative. Then the contents of 20 ticks were injected into the jugular vein, and examinations made in 10 minutes and in 20 minutes. Other examinations were made Sept. 29th and 30th, Oct. 2nd, 5th, 10th, 17th, 23rd and Nov. 2nd. No flagellates were found.

On October 2nd the contents of the digestive tracts of 25 ticks were injected subcutaneously into lambs 507, 572 and 606. They were examined Oct. 3rd, 5th, 10th, 17th and 23rd, but no flagellates appeared in the blood. On Oct. 28th lamb 507 was killed. In the case of birds Woodcock (1910) was able to find trypanosomes in the bone marrow. altho none could be found in the peripheral circulation. And so I determined to examine the bone marrow, and the blood from the lungs, the spleen and the liver of this lamb. To make sure whether flagellates were present, I examined these organs in fresh preparations and in stained smears. No flagellates were found, altho this lamb had been for months heavily infected with ticks containing *C. melophagia* and had been inoculated twice and fed once with macerated ticks. Lambs 572 and 606 were examined again Nov. 1st with negative results.

The last method consisted in eliminating the possibility of young ticks obtaining flagellates by ingesting faeces from infected ticks. This was accomplished as follows: Pupae were collected and washed in several changes of water to remove any faecal matter which might be adhering. They were then placed in a clean box and allowed to hatch. A lamb was thoroughly washed in a soapy antiseptic dip and rinsed with water to kill or remove all parasites present in deposited faecal matter. The young ticks were then placed upon this lamb, (No. 606).

Owing to the fact that a great many of the young died before feeding more than a couple of times, only eleven ticks reached an age of about two weeks or over. Young ticks before feeding several times have never shown flagellated forms of *Crithidia,* altho in years past as well as at this time, I have examined them for flagellates. [Nevertheless, the parasites are present as has been shown by Miss Porter (1910).] For example, a young tick which was examined October 14th after it had fed three times—Oct. 7th, 10th and 12th—showed no flagellated forms. Another which was examined three days after its first feeding contained no flagellated forms. But, two of those, which reached further development, were examined 12 days after they were put on the lamb, and were found to be teeming with flagellates. Also nine others, which were between two and three weeks old when they were examined, were packed full of flagellates.

Thus it is evident that the flagellated forms of the *Crithidia* develop when the ticks are between one and three weeks old. Since it was impossible for these young ticks to ingest faeces from infected ticks, their flagellates must either have been obtained from the blood of the lamb, or have been inherited. I conclude the latter, otherwise it would have been possible to have found them in the blood of the lamb. In a former paper (1909) I claimed that heriditary transmission exists, having found parasites in the ova. Later Miss Porter (1910) confirmed this observation and even traced the parasite in the pupae and young ticks. These facts together with the above experiment justify the conclusion which I have made.

From this experiment it appears that hereditary infection is very general, since the eleven ticks, which reached sufficient development, all showed flagellates. In my paper (1909) I held that hereditary transmission must be the most common mode of infection, and these experiments confirm the idea. Ticks must be over three weeks old before they lay their first pupae. Therefore, since the flagellates develop before the ticks are three weeks old, their first offspring as well as the later ones are exposed to hereditary infection.

To sum up, the contents of the digestive tracts of eighty-six ticks were injected subcutaneously, intraperitoneally and intravenously into four lambs. In every case the injected fluid contained great numbers of parasites of different shapes. Besides these injections each lamb was fed on ticks together with their excrement. In all, over sixty examinations of these lambs' blood were made, the results always being negative. If *Crithidia melophagia* is communicable to the sheep, it would indeed seem strange that infection was not accomplished when all the conceivable avenues of possible infection were tried. It must also be remembered that these lambs were also heavily infested with sheep ticks containing flagellates, practically all the time the experiments were running. And so, tho infection might not be possible by inoculation and feeding of the contents of sheepticks, it ought to have taken place by the normal associations of the ticks with the lambs, provided the flagellate is communicable to the sheep. In the light of this experimental evidence, it must be concluded that the flagellate of the tick is not communicable to the sheep and hence that it is not the insectan phase of the life-cycle of a sheep trypanosome. Evidence has also been produced showing that the flagellate is transmitted hereditarily from mother-tick to offspring. And Dr. Porter (1910) has shown that it passes from one tick to another by the ingestion of faeces. Thus it is not necessary to the propagation and spread of the species, for it to pass thru the blood of the sheep.

In closing, certain other arguments against Woodcock's view might be presented advantageously. For example, since practically all ticks over two weeks old (that is, after they have sucked blood a half dozen times) and collected from any sheep or lamb in the flock contain *Crithidia*, these flagellates must be very widely distributed among the sheep and must be fairly numerous in their blood, provided they are merely sheep trypanosomes taken up by the tick in the act of sucking blood. Some one might suggest that they are trypanosomes so small in the blood as to escape notice. But this cannot be, for Woodcock in describing the trypanosome, which he claims is connected with *Crithidia melophagia*, says it is a "typical, active trypanosome." It is unfortunate that he did not give any details of the methods used in discovering this trypanosome. He says that "after prolonged examination of the blood of a sheep on which were 'keds' infected with this parasite" he found the trypanosome. He does not state how many examinations he made nor over how long a period his examinations extended. Therefore it is impossible to say whether the examinations I have made were as prolonged and numerous as his. It is evident, however, that I made enough examinations to discover the trypanosome, provided it is so prevalent that ticks are able to take it up in the few small drops of blood they would suck in a couple of weeks; and such must be the case, provided their flagellates are derived from a trypanosome in the sheep's blood, since the ticks all become infected within two or three weeks.

Against Woodcock's view I would mention the fact that a trypanosome has never been found in the digestive tract of the tick, altho hundreds of examinations have been made both of ticks infected and uninfected with *Crithidia*. Yet, in the case of rat-fleas fed on rats infected with trypanosomes, it is very easy to find typical trypanosomes even in the intestine long after all the flagellates have disappeared from the stomach. If *C. melophagia* is the insectan phase of a trypanosome cycle, we would expect occasionally to find at least in *young* ticks a trypanosome form.

In view of the evidence presented in this paper and by other investigators, I am unable to accept the idea that *C. melophagia* is a developmental phase in the life-cycle of a sheep trypanosome, and much more unable, the generalization that "*Crithidia* and *Herpetomonas* from *all blood-sucking* insects are merely developmental phases of some Trypanosome." If any one continues to hold this view the *onus probandi* certainly lies with him. Such a view should not be asserted by anyone, unless he is willing to accept the *onus probandi* and actually prove the view by experiment. No view can be considered scientific and reasonable when it is opposed by experimental evidence, no matter how plausible it may appear on *a priori* grounds. Were this not true, we would discard all experimental laboratories.

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